

DATA EVALUATION RECORD
WHOLE SEDIMENT ACUTE TOXICITY, FRESHWATER INVERTEBRATES
OPPTS Guideline 850.1735

1. **CHEMICAL:** Cyfluthrin PC Code No.: 128831

2. **TEST MATERIAL:** Cyfluthrin technical Purity: 94.10%

3. **CITATION:**

Authors: Picard, C.R.
Title: 10-Day Toxicity Test Exposing Midge (*Chironomus dilutus*)
to Cyfluthrin Applied to Formulated Sediment Under Static-
Renewal Conditions Following OPPTS Draft Guideline
850.1735.

Study Completion Date: June 30, 2010

Laboratory: Springborn Smithers Laboratories
790 Main Street
Wareham, MA 02571-1037

Sponsor: Pyrethroid Working Group
Beveridge & Diamond
1350 I Street NW
Washington, DC 20005

Laboratory Report ID: 13656.6144

MRID No.: 48593607

DP Barcode: 397235

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, CSS-Dynamac Corporation

Signature: 

Date: 11/19/12

APPROVED BY: Teri S. Myers, Environmental Scientist, CDM Smith

Signature: 

Date: 09/30/13

5. **APPROVED BY:** Tanja Crk

Signature: 

Date: 05/25/16

6. **STUDY PARAMETERS:**

Test Organism:	Dipteran midge, <i>Chironomus dilutus</i>
Age of Test Organism:	3 rd Instar, 10 to 11 days old
Definitive Test Duration:	10 days
Study Method:	Flow-through
Type of Concentrations:	Mean-measured sediment, bulk and OC-normalized

7. CONCLUSIONS:

Results Synopsis (from CETIS):

Based upon mean-measured sediment concentrations:

Survival:

LC₅₀: 88 µg ai/kg 95% C.I.: 68 to 106 µg ai/kg
Probit Slope: 3.24 (2.39 to 4.11)
NOAEC: <14 µg ai/kg
LOAEC: 14 µg ai/kg

Growth (AFDW):

EC₅₀: >250 µg ai/kg 95% C.I.: N/A
NOAEC: 14 µg ai/kg
LOAEC: 27 µg ai/kg

Based upon OC-normalized mean-measured sediment concentrations:

Where $f_{oc} = TOC = 2.3\% = 0.023$

Survival:

LC₅₀: 3,826 µg ai/kg TOC 95% C.I.: 2,957 to 4,609 µg ai/kg TOC
NOAEC: <609 µg ai/kg TOC
LOAEC: 609 µg ai/kg TOC

Growth (AFDW):

EC₅₀: >10,870 µg ai/kg TOC 95% C.I.: N/A
NOAEC: 609 µg ai/kg TOC
LOAEC: 1,174 µg ai/kg TOC

Calculated freely dissolved pore water concentrations (from: mean-measured OC-normalized/ ([K_{oc}])

Where $K_{oc} = 184,864 \text{ mL/g}_{oc}$

Survival:

LC₅₀: 0.02 µg ai/L 95% C.I.: 0.016 to 0.025 µg ai/L
NOAEC: <0.003 µg ai/L
LOAEC: 0.003 µg ai/L

Growth (AFDW):

EC₅₀: > 0.059 µg ai/L 95% C.I.: N/A
NOAEC: 0.003 µg ai/L
LOAEC: 0.006 µg ai/L

8. ADEQUACY OF THE STUDY:**A. Classification:** Acceptable**B. Rationale:** N/A**C. Repairability:** N/A**9. MAJOR GUIDELINE DEVIATIONS:**

There were no deviations from OPPTS 850.1735 guidance that would affect the scientific soundness or acceptability of this study.

10. MATERIALS AND METHODS:**A. Test Organisms**

Guideline Criteria	Reported Information
Species: <i>H. azteca</i> or <i>Chironomus tentans</i>	Dipteran midge, <i>Chironomus dilutus</i>
Life Stage: For <i>C. tentans</i> : third instar (9-11 days old). The instar stage of midges must be confirmed by head capsule width (approx. 0.38 mm). For <i>H. azteca</i> : 7- to 14-day old amphipods must be produced. If growth is also an endpoint, a narrower range, such as 1- to 2-day old amphipods should be used.	3 rd instar, 10 days old At study initiation, the head capsule width of a sub-population of 20 larvae ranged from 0.30 to 0.45 mm (mean of 0.38 mm), and the dry weight of a sub-population of 20 larvae averaged 0.19 mg/larvae.
Supplier Brood stock can be obtained from laboratory, commercial, or government sources. (Sources obtained from the wild should be avoided unless cultured through several generations in the laboratory.)	Environmental Consulting & Testing Superior, WI
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period: The required culture and testing temperature is 23°C. The test organisms should be cultured in the same water to be used for testing.	Midges were acclimated to test temperature for <i>ca.</i> 48 hours prior to test initiation. During the holding period, the water temperature was maintained at 22°C and the dissolved oxygen ranged from 7.4 to 8.3 mg/L.
Feeding:	Finely-ground flaked fish food suspension daily.
Pretest Mortality: A group of organisms should not be used if they appear unhealthy, discolored (eg <20% mortality 48 h before the beginning of a test).	0% mortality in the 24 to 48 hours prior to test initiation.

C. Test System

Guideline Criteria	Reported Information
<p>Source of dilution water (overlying water) and sediment: Soft reconstituted water or water from a natural source. Tap water is acceptable if it is dechlorinated, deionized, and carbon filtered, but its use is not encouraged.</p> <p>Uncontaminated natural sediment is recommended.</p>	<p>Laboratory well water characterized as having a total hardness and total alkalinity as CaCO₃ of 60 to 78 mg/L and 22 to 24 mg/L, respectively, a pH range of 6.9 to 7.0, and a specific conductivity range of 440 to 460 µmhos/cm.</p> <p>Formulated sediment (Springborn Smithers Batch No. 112509A) was prepared according to OECD Guideline 218 by mixing the following components (dw basis): 6.0% sphagnum peat, 20% kaolin clay, and 74% fine sand. While blending using a large-scale mixer, 6 L of laboratory well water was also added.</p> <p>Prior to use, the sphagnum peat was pre-soaked in dilution water for 5 days. During this time, the peat was amended with 142 g of powdered CaCO₃ to increase the pH from 3.2 to 6.0.</p>
<p>Does water support test animals without observable signs of stress?</p>	<p>Yes</p>
<p>Quality Of Water If problems are observed in culturing or testing of organisms, it is desirable to test water quality. Particulate, TOC, COD should be <5 mg/L and residual chlorine <11 µg/L</p>	<p>There were no apparent problems with water quality.</p> <p>On Day 0, the measured ammonia level (as N) in control sediment pore water was 16 mg/L.</p>

Guideline Criteria	Reported Information
Water Temperature 23°C for both species. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1°C and 3°C, respectively.	Daily: 22 to 24°C Continuous: 22 to 25°C
pH Should not vary more than 50%. Survival is best at pH >6.5 for <i>C. tentans</i> .	6.8 to 7.4
Dissolved Oxygen Maintained between 40 and 100%.	3.5 to 7.9 mg/L (>40% saturation)
Total Hardness Should not vary more than 50%. <i>H. azteca</i> are sensitive to hardness (e.g., they are not found in waters with calcium at <7 mg/L and DO at <2 mg/L).	60 to 76 mg/L as CaCO ₃
Conductivity Should not vary more than 50%.	370 to 400 µmhos/cm
Sediment Characterization All sediment must be characterized for: pH, ammonia concentration of pore water, organic carbon content (total organic carbon (TOC)), particle size distribution, and percent water content.	Particle distribution – 79% sand, 6% silt, 15% clay (sandy loam; reviewer-derived from USDA soil texture triangle) TOC – 2.3% Percent solids – 63.74% pH – 7.1 CEC – not reported Bulk density – not reported
Additional Sediment Analysis BOD, COD, cation exchange capacity, Eh, pE, total inorganic carbon, total volatile solids, acid volatile sulfides, total ammonia, metals, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis.	Not reported

Guideline Criteria	Reported Information
<p>Laboratory Spiked Sediment</p> <p>Material should be reagent grade unless prior evaluations dictate formulated materials, etc.; Must know the test material's identity, quantity of major ingredients and impurities, water solubility, estimated toxicity, precision and bias of analytical method, handling and disposal procedures.</p>	<p><u>Cyfluthrin technical</u></p> <p>IUPAC name: (RS)-α-cyano-4-fluoro-3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate</p> <p>CAS name: cyano(4-fluoro-3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate</p> <p>Description: not reported</p> <p>Batch no.: EBCTFK103</p> <p>CAS No.: 68359-37-5</p> <p>Purity: 94.10%</p> <p>Storage: room temperature in the dark</p> <p>Aqueous solubility: not reported</p>
<p>Stock Solutions</p> <p>Test material should be dissolved in a solvent prior to mixing into test sediment; If solvent is used, both solvent control and negative control are required.</p>	<p>A 100-μg ai/mL primary stock solution was prepared by dissolving 0.01061 g test substance (0.01000 g ai) into 100 mL of acetone.</p> <p>Six individual dosing solutions were prepared by diluting the appropriate amount of the primary stock solution (0.660 to 20.6 mL) into 25 mL acetone.</p> <p>All dosing solutions were clear and colorless, with no visible un-dissolved test substance.</p> <p>Negative and solvent controls were included in the test.</p>

Guideline Criteria	Reported Information
<p>Test Concentrations For Spiked Sediment For LC50 calculation, test concentrations should bracket the predicted LC50; sediment concentrations may be normalized to factors other than dry weight (e.g. organic content, acid volatile sulfides); Sediment may be mixed using rolling mill, feed mixer or hand mixer.</p>	<p>A jar-rolling technique was used to apply the test substance to the sediment. A 10-mL volume of the appropriate prepared dosing stock solution (in acetone) was applied to 0.0500 kg of fine silica sand and the solvent was allowed to evaporate off for 45 minutes. The dry sand was then added to 2.5 kg of wet sediment (total of 1.6435 kg dw) in individual glass jars. Each jar was then rolled for 4 hours at <i>ca.</i> 15 rpm. The jars were stored upright at 2 to 8°C for a 14-day equilibration period.</p> <p>Twice a week during the equilibration period and prior to being added into the replicate exposure vessels, the jars were mixed on the rolling mill for 2 hours to ensure the sediment was homogeneous.</p> <p>The range of nominal concentrations (16 to 500 µg ai/kg dw) was based upon the results of a preliminary range-finding study.</p>
<p>Test Aquaria 1. <u>Material</u>: Glass or stainless steel or perfluorocarbon plastics. 2. <u>Size</u>: 300 ml high-form lipless beakers containing 100 ml of sediment and 175 ml of overlying water.</p>	<p>1. Glass and 40-mesh Nitex screen (for drainage) 2. 300 mL vessels containing 100 mL (<i>ca.</i> 4.0-cm layer) of sediment (equivalent to 98.2 g dw) and 175 mL of overlying water. The total overlying water plus sediment volume was maintained at <i>ca.</i> 275 mL.</p>
<p>Type of Dilution System Daily renewal or a flow-through system may be used.</p>	<p>Flow-through</p>
<p>Flow Rate 2 volume changes/day</p>	<p>2 volume additions/day</p>

Guideline Criteria	Reported Information
Aeration Dilution water should be vigorously aerated prior to use so that dissolved oxygen in the overlying water remains above 40% saturation.	None reported
Photoperiod 16 hours light, 8 hours dark at 500 to 1000 lux.	16 hours light, 8 hours dark; 590 to 990 lux
Solvents Use of a solvent should be avoided since they may influence the concentration in pore water. If used, it should not exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents include triethylene glycol, methanol, ethanol, or acetone. Surfactants should not be used.	Acetone, 10 mL per 1.6435 kg dw sediment It was reported that the acetone was allowed to completely evaporate during the mixing procedure.

D. Test Design

Guideline Criteria	Reported Information
Sediment Into Test Chambers One day prior (Day -1) to start of test: test sediment, reference sediment, and negative control sediment should be thoroughly homogenized and added to test chambers; Overlying water is added to chambers in a manner that minimizes suspension of sediment.	One day prior to the addition of chironomid larvae (day -1), the test systems were established. Overlying water was gently added, and each vessel was placed under the renewal system.

Guideline Criteria	Reported Information
<p>Renewal of Overlying Water: Renewal of the overlying water should be conducted on day -1 prior to the addition of organisms or food on day 0. For flow-through systems, the flow rates should not vary by more than 10% between any two chambers at any time. Proper operation should be verified by calibration prior to test initiation.</p>	<p>The overlying water was renewed via an intermittent delivery system in combination with a calibrated water-distribution system. The test system was calibrated before and after the test, and visually inspected at least twice daily for proper functioning.</p>
<p>Placing Organisms in Test Chambers: Should be handled as little as possible and introduced into overlying water below the air-water interface.</p>	<p>Midges were impartially assigned one or two at a time into intermediate test beakers until all beakers contained ten midges. The test was initiated when each intermediate beaker of midges was added to each respective test vessel.</p>
<p>Range Finding Test A definitive test will not be required if no toxicity is observed at concentrations of 100 mg/kg dry weight of sediment.</p>	<p><u>Preliminary toxicity assessment</u></p> <ul style="list-style-type: none"> • 10-day exposure at nominal levels of 0 (negative and solvent controls), 0.20, 2.0, 20, 200, and 2000 µg ai/kg • 9-day old larvae; three replicates per level, each containing 10 larvae • Survival averaged 83 (control), 93 (solvent control), 87, 87, 93, 30, and 0%, respectively • AFDW averaged 0.87 (control), 0.76 (solvent control), 0.89, 0.74, 0.69, and 0.25 mg per larva, respectively
<p>Monitoring the test All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance.</p>	<p>Test vessels were observed daily for mortality and abnormal behavior.</p>
<p>Nominal Concentrations of Definitive Test Control(s) and at least 5 test concentrations; dilution factor not greater than 50%. Concentrations above aqueous solubility may be used.</p>	<p>0 (negative and solvent controls), 16, 31, 63, 130, 250, and 500 µg ai/kg dw</p>

Guideline Criteria	Reported Information
<p>Number of Test Organisms 10 organisms per test chamber are recommended. 8 replicates per treatment should be used.</p>	<p>80 larvae per level, with 10 larvae per replicate vessel and 8 biological replicates per level</p> <p>An additional 6 replicates per level were maintained for chemical analysis and pore water quality and analysis. The additional replicates contained organisms.</p>
<p>Test organisms randomly or impartially assigned to test vessels?</p>	<p>Yes</p>
<p>Feeding <i>C. tentans</i> in each test chamber are fed 1.5 ml of a 4 g/L Tetrafin⁷ suspension daily. <i>H. azteca</i> may be fed with a mixture of yeast, Cerophy., and trout chow (YCT) at a rate of 1.5 mL daily per test chamber. A drop in DO. levels below 2.5 mg/L may indicate over-feeding and feeding should be suspended in all treatments until DO levels increase.</p>	<p>Midges were fed a finely-ground flaked fish food suspension (4.0 mg/mL) once daily at a rate of 1.5 mL/vessel.</p>
<p>Water Parameter Measurements Conductivity, hardness, pH, alkalinity, and ammonia should be measured in all treatments at the beginning and end of the test.</p> <p>DO should be measured daily.</p> <p>Temperature should be measured daily in one test chamber from each treatment. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1 and 3°C, respectively.</p>	<p>Total hardness, alkalinity, specific conductance, and ammonia were measured in each treatment level and control solution from a composite sample at Days 0 and 10.</p> <p>Dissolved oxygen (DO), temperature, and pH were measured in each replicate vessel on Days 0 and 10, and in one alternating replicate from each level on Days 1 to 9. In addition, the temperature was continuously monitored in an auxiliary vessel in the temperature-controlled water bath.</p>

Guideline Criteria	Reported Information
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used. Concentrations should be measured in bulk sediment, interstitial water, overlying water, and stock solution.	Sediment from all levels was analyzed for cyfluthrin on Days 0 and 10. Following removal of the overlying water, the sediment was centrifuged at <i>ca.</i> 1200 g for 15 to 30 minutes and extracted and analyzed using GC/MS based on methodology validated at Springborn Smithers (see Reviewer's Comments section for further details).

11. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes (see Reviewer's Comments).
Control Criteria Was control mortality $\leq 30\%$? Were control <i>C. tentans</i> an average size of ≥ 0.6 mg?	Negative control: 14% Solvent control: 16% Negative control: 1.26 mg/larva Solvent control: 1.29 mg/larva
Percent Recovery of Chemical:	Results of quality control (QC) samples analyzed concurrently with test samples: <u>Sediment:</u> 88.0 to 105% of nominal

Guideline Criteria	Reported Information
Data Endpoints <ul style="list-style-type: none">- Survival- Dry weight (determined by pooling all living organisms from a replicate and drying at 60 to 90°C to a constant weight)- Body length (amphipod only)	<ul style="list-style-type: none">- Survival- Ash-free dry weight (AFDW)
Raw data included?	Yes, sufficient

Effects Data

Toxicant Concentration			Survival		Ash-Free Dry Weight (AFDW) per Larva		
Nominal ($\mu\text{g ai/kg}$)	Mean-Measured ^(a)						
	Sediment ($\mu\text{g ai/kg}$)	Pore Water ($\mu\text{g ai/L}$)	Overlying Water ($\mu\text{g ai/L}$)	% \pm SD	% Inhibition ^(b)	mg \pm SD	% Inhibition ^(b)
Negative Control	<LOQ ^(c)	Not reported	Not assessed	86 \pm 14	N/A	1.26 \pm 0.37	N/A
Solvent Control	<LOQ	Not reported	Not assessed	84 \pm 18	N/A	1.29 \pm 0.24	N/A
Pooled Control	---	---	---	85 \pm 15	N/A	1.27 \pm 0.30	N/A
16	14	Not reported	Not assessed	74 \pm 21	13	1.18 \pm 0.27	7
31	27	Not reported	Not assessed	68 \pm 18*	20	0.83 \pm 0.15 ^(d)	35
63	55	Not reported	Not assessed	59 \pm 11*	31	1.30 \pm 0.34 ^(d)	-2
130	130	Not reported	Not assessed	28 \pm 10*	67	0.95 \pm 0.42 ^(d)	25
250	250	Not reported	Not assessed	4 \pm 5*	95	0.77 \pm 0.58 ^(d)	39
500	510	Not reported	Not assessed	0 \pm 0*	100	N/A	N/A

^(a) Results of the pore water analysis were not included in the study report and will be presented in a supplemental report, as stated by the study author. Overlying water was not analyzed in this study due to pyrethroids' strong affinity to sediment and regular renewal of the overlying water.

^(b) Relative to the pooled control.

^(c) LOQ = 1.2 $\mu\text{g ai/kg}$.

^(d) Excluded from statistical analysis due to significant effect on survival at this level.

* Statistically-significant compared to the pooled control ($p < 0.05$).

Other Significant Results:

Biological: After 10 days, survival averaged 86 and 84% for the negative and solvent control groups, respectively, compared to 74, 68, 59, 28, 4, and 0% for the mean-measured 14, 27, 55, 130, 250, and 510 $\mu\text{g ai/kg}$ levels, respectively. Differences were statistically significant ($p < 0.05$) compared to the pooled control (85%) at the ≥ 27 $\mu\text{g ai/kg}$ levels. Using mean-measured concentrations, the NOAEC and LOAEC for survival were 14 and 27 $\mu\text{g ai/kg}$, respectively, and the 10-day LC_{50} (with 95% C.I.) was 96 (83 to 110) $\mu\text{g ai/kg}$.

Ash-free dry weights (AFDW) averaged 1.26 and 1.29 mg per larva for the negative and solvent control levels, respectively, and 1.18, 0.83, 1.30, 0.95, and 0.77 mg per larva for the mean-measured 14, 27, 55, 130, and 250 $\mu\text{g ai/kg}$ levels, respectively. No statistically-significant difference was observed at the 14 $\mu\text{g ai/L}$ level compared to the pooled control (1.27 mg/larva). Higher treatment levels were not statistically assessed due to significant reductions in survival at these levels. Using mean-measured concentrations, the NOAEC and LOAEC for growth were 14 and >14 $\mu\text{g ai/kg}$, respectively, and the observed 10-day EC_{50} was >250 $\mu\text{g ai/kg}$.

Analytical: Only results from sediment analyses were reported in this study. Cyfluthrin concentrations decreased slightly at all levels during the 10-day study (-20 to -3.6% of Day-0 measurements, reviewer-calculated). Overall, mean-measured concentrations represented 86 to 101% of nominal sediment concentrations.

B. Statistical Results

Statistical analyses were performed on midge survival and growth (ash-free dry weight, AFDW). Analyses were performed using the response values for each replicate test vessel within a treatment level. Percent survival data were arcsine square-root transformed prior to analysis.

A t-Test was used to compare the performance of the negative control and solvent control data. For both endpoints, data were statistically similar, and the treatment groups were compared to the pooled control data to determine potential treatment-related effects.

Normality of the data was evaluated using the Chi-Square Test and homogeneity of variance was evaluated using Bartlett's or Cochran's Tests at the 99% level of certainty. Survival and growth data met both assumptions and were therefore analyzed using Bonferroni's t-Test (at a 95% level of certainty). Due to the statistical effect on survival in all but one concentration, a two-sample t-Test was also applied to establish treatment effects for the growth endpoint. NOAEC and LOAEC values were assigned based upon significance.

Probit Analysis was used to calculate the LC_{50} value (survival) with associated 95% confidence intervals. Analyses were performed using TOXSTAT Version 3.5 statistical software and mean-measured sediment concentrations.

Survival:LC₅₀: 96 µg ai/kg

95% C.I.: 83 to 110 µg ai/kg

NOAEC: 14 µg ai/kg

LOAEC: 27 µg ai/kg

Growth (AFDW):EC₅₀: >250 µg ai/kg

95% C.I.: N/A

NOAEC: 14 µg ai/kg

LOAEC: >14 µg ai/kg

12. VERIFICATION OF STATISTICAL RESULTS:Statistical Method:

The reviewer tested for normality using the Shapiro-Wilk test for normality and for homogeneity of variance using the Bartlett test for equality of variance or Levene's Test for Equality of Variance. Both growth (as ash-free dry weight) and survival met the assumptions of normality and homogeneity of variance. As a result, both end points were analyzed using the Dunnett Multiple Comparison test ($p < 0.05$). Survival exhibited a linear, decreasing dose response, therefore results of statistical analysis using William's Multiple Comparison Test are reported. Significant decreases were noted at every treatment level for survival relative to the negative control, and 100% mortality was observed in the highest dose treatment group. A dose-dependent response was not observed for the growth endpoint, although a significant decrease was observed for the 27 µg a.i./kg mean-measured sediment treatment group. An LC₅₀ for survival was reported using the Probit slope analysis and an EC₅₀ for growth was attempted using nonlinear regression ("Bruce and Versteeg" model); however, the 95% confidence interval was not calculable, so this estimate was empirically estimated because inhibition did not approach 50%.

The negative and solvent control groups were compared using a Student's t-test assuming equal variances; no significant difference was detected ($p > 0.05$) and subsequent treatment level comparisons were made to the negative control response only. These analyses were conducted using CETIS v. 1.8.7.12 with backend settings implemented by EFED on 5/29/13.

13. REVIEWER'S COMMENTS:

The reviewer's conclusions differed from the study author's. The study author reported no significant differences from the pooled control for growth; however the reviewer observed a significant reduction from the negative control at the 27 µg a.i./kg mean-measured sediment treatment group. The LC₅₀ for survival was calculated by the reviewer as 88 µg ai/kg with a C.I. of 68 to 106. This value was less than the study author's calculated value of 96 µg ai/kg. Comparison of the treatment levels to the negative control only, rather than a pooled control, may have contributed to the reviewer's differing conclusion from the study author.

Results were provided in terms of mean-measured sediment (bulk and OC-normalized) in the Conclusions section of the DER. Overlying water was not analyzed due to the pyrethroids' strong affinity to sediment (i.e., high K_{oc} values) and regular renewal of the overlying water. Documentation supporting that only negligible amounts of pyrethroids partition to overlying water were cited (Springborn Smithers Laboratories Study Nos. 13656.6106, 13656.6107, 13656.6110, 13656.6111, and 13656.6112; Putt, 2005). It was reported that data for cyfluthrin concentrations in pore water and bulk sediment for pore water will be presented in a supplemental report.

Dosing stock solutions and treated sediment from all levels (prior to allocation into the replicate vessels) were analyzed for cyfluthrin. Recoveries in the stock solutions ranged from 100 to 160% of nominal concentrations. Analysis of the spiked sediment following dosing and prior to allocation into the replicate exposure vessels ranged from 82 to 91% of nominal concentrations.

The analytical method used to quantify cyfluthrin in formulated sediment was validated on April 30 to May 5, 2009. Fortified samples were extracted two to three times with methanol:purified reagent water and hexane; the extracts were combined and purified for analysis using solid phase extraction (SPE). Aliquots were analyzed using gas chromatography equipped with mass selective detection in negative chemical ionization mode (GC-MS/NCI). The method validation established an average recovery of $92.6 \pm 5.43\%$ (CV=5.87%) for cyfluthrin from formulated sediment fortified at 0.100 and 100 µg ai/kg. The limit of quantitation (LOQ) was 0.0527 µg ai/kg. A method validation extension was conducted in February 2010, which established an average recovery of $98.4 \pm 2.99\%$ (CV=3.04%) for cyfluthrin from formulated sediment fortified at 2000 µg ai/kg.

In addition to total hardness and specific conductivity, total alkalinity and ammonia were determined in the overlying water of each level on Days 0 and 10. Total alkalinity ranged from 18 to 24 mg/L as CaCO₃, and ammonia (as N) ranged from 0.16 to 2.0 mg/L.

The redox potential, pH, ammonia content, and dissolved organic carbon (DOC) were measured in isolated pore water at each level on Days 0 and 10. The total organic carbon (TOC) was also measured in isolated pore water on Day 10, but inadvertently not measured on Day 0. The redox potential ranged from 190 to 210 mV, the pH ranged from 6.8 to 7.0, TOC and DOC were 110 to 140 mg C/L and 84 to 140 mg C/L, respectively, and the ammonia content (as N) ranged from 14 to 16 mg/L on Day 0 and from 3.9 to 6.1 mg/L on Day 10.

Overlying water temperature on Day 9 was 25°C (as measured by the minimum/maximum thermometer), exceeding the recommended $23\pm1^{\circ}\text{C}$ range. It was reported that since the temperature remained within the tolerance range for the test organism, the deviation did not impact the results of the study.

This study was conducted in compliance with all pertinent U.S. EPA GLP regulations (40 CFR, Part 160) with the following exceptions: routine water, sediment, and food contaminant screening analyses. These analyses were performed using certified laboratories and standard validated methods.

Definitive test dates were January 29 to February 8, 2010.

14. REFERENCES:

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CETIS Summary Report

Report Date: 30 Sep-13 21:36 (p 1 of 2)
 Test Code: 397235 48593607 | 19-3670-5313

OPPTS 850.1735 Sub-Chronic Sediment (10-d FW)

Springborn Smithers

Batch ID:	08-2206-8293	Test Type:	Sediment Toxicity 10-d	Analyst:	
Start Date:	29 Jan-10	Protocol:	OPPTS 850.1735 Sub-chronic Sediment (1	Diluent:	Well Water
Ending Date:	08 Feb-10 15:44	Species:	Chironomus dilutus	Brine:	Not Applicable
Duration:	10d 16h	Source:	Environmental Consulting & Testing	Age:	10 d

Sample ID:	07-7837-9625	Code:	48593607	Client:	EPA OCSPP EFED
Sample Date:	29 Jan-10	Material:	Cyfluthrin	Project:	Insecticide
Receive Date:		Source:	Pyrethroid Working Group		
Sample Age:	NA	Station:			

Batch Note: MRID 48593607

Sample Note: MRID 48593607

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
20-0490-0316	Ash Free Dry Weight	0	>0		26.5%		Equal Variance t Two-Sample Test
13-2845-7313	Ash Free Dry Weight	14	27	19.44	43.0%		Dunnett Multiple Comparison Test
19-8493-4182	Survival	0	>0		19.9%		Equal Variance t Two-Sample Test
15-8748-2565	Survival	<14	14	NA	14.7%		Williams Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/kg sed	95% LCL	95% UCL	TU	Method
19-7976-2504	Ash Free Dry Weight	IC50	1440	N/A	N/A		Nonlinear Regression
09-1885-2090	Survival	LC50	88	68.2	106		Linear Regression (MLE)
18-7553-9088	Survival	LC50	76	63.3	91.3		Trimmed Spearman-Kärber

Ash Free Dry Weight Summary

C-µg/kg sed	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	8	1.28	1.08	1.48	1.03	1.72	0.084	0.238	18.5%	0.0%
0	Negative Control	8	1.26	0.948	1.56	0.97	2.01	0.13	0.369	29.4%	2.05%
14		8	1.18	0.957	1.41	0.78	1.56	0.096	0.271	22.9%	7.7%
27		8	0.833	0.706	0.959	0.63	1.03	0.0534	0.151	18.1%	35.1%
55		8	1.3	1.02	1.58	0.88	1.88	0.118	0.335	25.7%	-1.56%
130		8	0.951	0.598	1.3	0.32	1.77	0.15	0.423	44.5%	25.8%
250		3	0.767	-0.674	2.21	0.12	1.24	0.335	0.58	75.6%	40.2%

Survival Summary

C-µg/kg sed	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	8	0.838	0.69	0.985	0.5	1	0.0625	0.177	21.1%	0.0%
0	Negative Control	8	0.863	0.745	0.98	0.6	1	0.0498	0.141	16.3%	-2.99%
14		8	0.738	0.565	0.91	0.4	1	0.073	0.207	28.0%	11.9%
27		8	0.675	0.528	0.822	0.4	1	0.062	0.175	26.0%	19.4%
55		8	0.588	0.493	0.682	0.4	0.7	0.0398	0.113	19.2%	29.9%
130		8	0.275	0.188	0.362	0.1	0.4	0.0366	0.104	37.6%	67.2%
250		8	0.0375	0	0.0808	0	0.1	0.0183	0.0518	138.0%	95.5%

CETIS Summary Report

Report Date: 30 Sep-13 21:36 (p 2 of 2)
Test Code: 397235 48593607 | 19-3670-5313

OPPTS 850.1735 Sub-Chronic Sediment (10-d FW)

Springborn Smithers

Ash Free Dry Weight Detail

C-µg/kg sed	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Solvent Blank	1.05	1.72	1.46	1.36	1.36	1.17	1.03	1.11
0	Negative Control	1.07	1.06	1.35	2.01	1.57	1	0.97	1.02
14		1.49	1.11	1.32	0.9	1.56	1.08	0.78	1.23
27		0.65	1.03	0.96	0.96	0.63	0.77	0.9	0.76
55		1.88	1.31	1.42	1.43	1.5	1.1	0.9	0.88
130		1.08	1.08	0.32	1.77	0.79	0.65	0.83	1.09
250		0.12			1.24	0.94			

Survival Detail

C-µg/kg sed	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	Solvent Blank	1	0.5	0.9	1	1	0.7	0.8	0.8
0	Negative Control	1	0.9	0.7	0.6	0.9	0.9	1	0.9
14		0.8	0.9	0.7	1	0.4	0.7	0.9	0.5
27		0.8	0.6	0.4	0.6	1	0.7	0.6	0.7
55		0.7	0.7	0.6	0.4	0.6	0.5	0.7	0.5
130		0.3	0.2	0.1	0.2	0.3	0.4	0.3	0.4
250		0.1	0	0	0.1	0.1	0	0	0